

Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Related Chemicals

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Introduction

Chlorinated dibenzo-*p*-dioxins have been identified as trace contaminants of herbicides and pesticides. They may be formed as by-products in the manufacture of chlorinated phenols and, for this reason, were implicated in outbreaks of chick edema disease (1). Fat trimmings obtained from animal hides preserved with dioxin-containing chlorophenol products and subsequently used in chicken feed contained the chick edema factor. Chloracne had been earlier observed in workers who had handled technical quality 2,4,5-trichlorophenol (2). Occasionally the condition was complicated by liver involvement and psychopathological changes. Pure 2,4,5-trichlorophenol had no effect when tested in an animal bioassay system. Tetrachlorodibenzodioxin (TCDD), which was isolated from the technical mixture, produced an effect at a concentration of 0.005%, however (13). In addition, TDCC is the contaminant of the herbicide 2,4,5-T that produced teratogenic effects in rats (4). Subsequent work with pure TCDD has indeed shown teratogenic and embryotoxic effects in rats and mice (5-7).

The fact that the chlorinated dioxins are highly toxic, teratogenic, and acnegenic does not of necessity indicate they are carcinogens

as well. However in view of the widespread use of products that might contain dioxins as contaminants and their extreme stability under environmental conditions, examination of their possible long-term effects is imperative. This program was initiated to determine the chronic toxicity and potential carcinogenicity of a series of chlorinated dibenzodioxins by oral administration and skin application. The unsubstituted, 2, 7-dichloro- and octachlorodibenzodioxins are relatively innocuous with regard to toxicity. The 2,3,7-trichloro-, 2,3,7,8-tetrachloro- and hexachlorodibenzodioxins, however, are quite toxic and require the use of specialized facilities to minimize the possibility of human exposure. This report describes our results to date with the nontoxic dioxins. Work is being initiated with the toxic dioxins as they become available.

Experimental

The octachlorodibenzodioxin used in these studies was prepared by Spectratec Inc., Washington, D. C. The other dioxins were synthesized by the Chemistry Division of IIT Research Institute. Swiss-Webster and B6C3F1 mice and Osborne-Mendel rats were obtained from contract supported colonies at Charles River Breeding Laboratories through arrangement with the Mammalian Genetics and Animal Production Section, National Cancer Institute.

Skin carcinogenesis studies will be continued for a total of 78 weeks. Each treatment

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group consists of 30 male and 30 female Swiss-Webster mice that are shaved weekly. In testing for complete carcinogenicity 0.2 ml of a solution of the test compound dissolved in acetone is applied three times weekly to the backs of mice. The octachloro-, dichloro-, and unsubstituted dibenzodioxin solutions in acetone contain 0.2, 3.0, and 80 mg/ml, respectively. For the study of promotion activity, each mouse was initially treated with 50 μ g dimethylbenzanthracene (DMBA) 1 week prior to initiation of test compound application.

Osborne-Mendel rats and B6C3F1 mice are being used in the oral administration studies. Groups of 50 male and 50 female mice and 35 male and 35 female rats are receiving the dioxins at levels of 1% and 0.5% of the diet, and similar groups are receiving 1% and 0.5% dioxane in their drinking water. In the case of the supposedly nontoxic octachlorodibenzodioxin, in which numerous animal deaths occurred after 20 weeks of feeding, additional animals were placed on test at lower dietary levels.

Results

The results for the skin carcinogenesis study to date are shown in Table 1. None of the dioxin-treated mice in the complete carcinogenesis study exhibited skin tumors, although subcutaneous tumors are present in two mice treated with octachlorodibenzodioxin. Histopathological results are not yet available for all mice that have died. Of those that have been examined, however, a malignant lymphoma of the lymphocyte type was found in a mouse treated with unsubstituted dioxin. No other significant lesions were found in any of the mice from the complete study.

The promotion study has yielded apparent skin tumors in male mice treated with unsubstituted dibenzodioxin or dichlorodibenzodioxin. As the animals showing these skin lesions are still alive, histologic confirmations of this observation is not yet available. A plasmacytoma has been found in one mouse from the dichlorodibenzodioxin promotion group.

Acetone was originally included in the complete study as a solvent control, since the dioxins are dissolved in it for skin application. No skin tumors have been found in either surviving mice or in tissue from those that have died. The acetone promotion groups, however, were started 3 months later, and skin tumors have been confirmed by microscopic examination of skin of a male mouse that died. A reticulum cell malignant lymphoma was found in one of the females that was examined, but there were no other significant lesions.

The dioxins can be considered as derivatives of 1,4-dioxane, and this compound was included in the studies because of the structural similarity. One carcinoma not microscopically confirmed and a subcutaneous tumor are apparent in the surviving mice of the complete study. Tissues from those that died revealed a reticulum cell malignant lymphoma but no evidence of skin lesions.

The dioxane promotion groups are of particular interest since their response unexpectedly rivaled that of the croton oil positive controls in both mortality and mice exhibiting papillomas. The activity of croton oil and dioxane as promoting agents following DMBA initiation is shown in Figures 1 and 2. Weekly counts of papillomas and suspect carcinomas were made by gross examination. The fraction of mice bearing skin tumors is nearly identical for both materials, as is the time course for tumor development (Fig. 1). However, croton oil treatment led to a much higher multiplicity of skin tumors per mouse than did treatment with dioxane (Fig. 2). Carcinomas were produced in both treatment groups in direct proportion to the number of papillomas present.

Histopathological results from the two treatments differed in that effects observed with croton oil were primarily neoplastic. A majority of the dioxane-treated mice had liver lesions of a mild nature (megaloctosis, occasional distended bile canaliculi, occasional necrotic centrilobular necrosis, cuffed triad vessels, general mononuclear periportal infiltration, and mild peripherolobular fibrosis). The distribution of preneoplastic and

Table 1. Summary of dioxin skin carcinogenesis data.

| Compound | Treatment | Week of test | Sex | Number | Survivors | | | | Pathological results |
|-----------------------------|-----------|--------------|-----|--------|----------------|----------------------|---|--------------------------------------|---|
| | | | | | Tumor response | | | Subcutaneous tumors | |
| | | | | | Papillomas | Suspected carcinomas | | | |
| Octachloro-dibenzodioxin | Complete | 60 | M | 20 | 0 | 0 | 1 | } | No papilloma or significant pathology |
| | | | F | 28 | 0 | 0 | 1 | | No papilloma or significant pathology |
| Unsubstituted dibenzodioxin | Promotion | 59 | M | 24 | 0 | 0 | 3 | } | No papilloma or significant pathology |
| | | | F | 29 | 0 | 0 | 1 | | No papilloma or significant pathology |
| | Complete | 59 | M | 24 | 0 | 0 | 0 | } | No papilloma, 1 malignant lymphoma |
| | | | F | 24 | 0 | 0 | 0 | | No papilloma or significant pathology |
| Croton oil | Promotion | 58 | M | 25 | 1 | 3 | 2 | } | No papilloma or significant pathology |
| | | | F | 29 | 0 | 0 | 0 | | No papilloma or significant pathology |
| | Promotion | 65 | M | 0 | — | — | } | Neoplastic lesions of skin and lungs | |
| | | | F | 1 | 0 | 1 | | 1 | No papilloma or significant pathology |
| Dichlorodibenzodioxin | Complete | 64 | M | 17 | 0 | 0 | 0 | } | No papilloma or significant pathology |
| | | | F | 27 | 0 | 0 | 0 | | No papilloma or significant pathology |
| | Promotion | 63 | M | 23 | 5 | 2 | 2 | } | No papilloma, 1 plasmacytoma |
| | | | F | 25 | 0 | 0 | 0 | | No papilloma or significant pathology |
| Acetone | Complete | 62 | M | 24 | 0 | 0 | 0 | } | No papilloma or significant pathology |
| | | | F | 28 | 0 | 0 | 0 | | No papilloma or significant pathology |
| | Promotion | 50 | M | 29 | 8 | 1 | 1 | } | 1 papilloma, 1 malignant lymphoma |
| | | | F | 26 | 0 | 0 | 0 | | No papilloma, 1 malignant lymphoma |
| Dioxane | Complete | 60 | M | 22 | 0 | 0 | 1 | } | No papilloma, 1 malignant lymphoma |
| | | | F | 25 | 0 | 1 | 0 | | No papilloma or significant pathology |
| | Promotion | 59 | M | 4 | 2 | 3 | 2 | } | Neoplastic lesions of skin, lungs, and kidney |
| | | | F | 5 | 2 | 3 | 0 | | No papilloma or significant pathology |

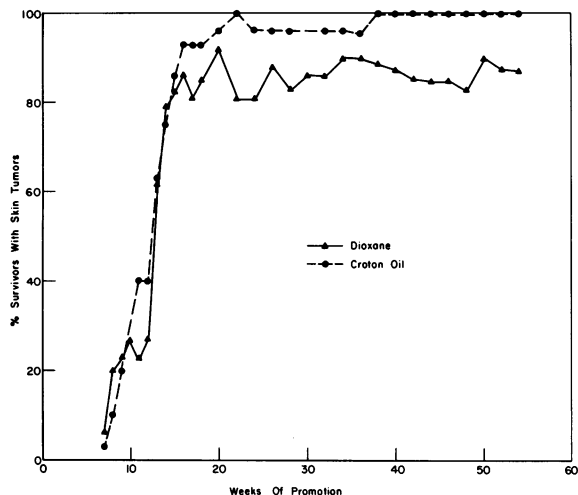


FIGURE 1. Comparative rate of promotion activity for croton oil and dioxane on male mice.

neoplastic lesions in the dioxane-treated group is shown in Table 2. In treated skin, conditions ranged from hyperplasia to dermal fibrosarcoma. Squamous cell carcinoma of the nasal septum was observed in one animal which had skin papilloma. Of nine mice with lung tumors, seven had malignant lung lesions, of which three were considered metastatic from other sites.

The average weights of controls and test animals receiving the dioxins in their diet and dioxane in water are shown in Figures

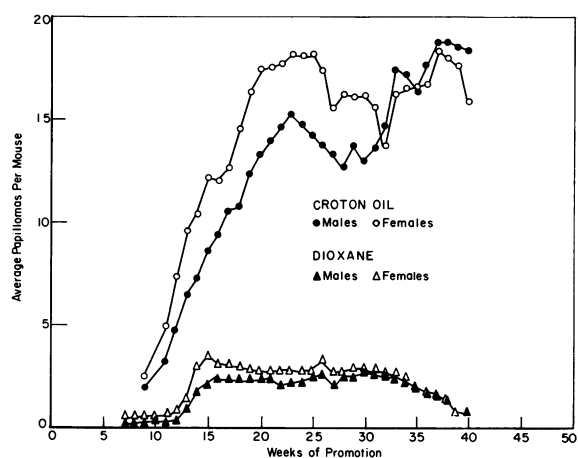


FIGURE 2. Promotion activity of 1% croton oil and dioxane on Swiss-Webster mice.

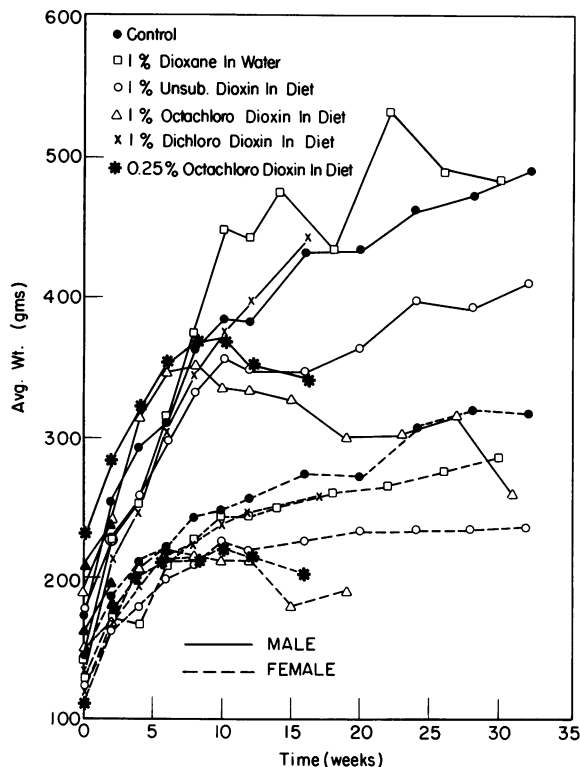


FIGURE 3. Average weight of treated female and male rats.

3 and 4 for rats and Figures 5 and 6 for mice. The stimulatory effect of dioxane on weight gain in male mice and rats is most interesting, especially in view of the skin carcinogenesis results. The effect is not as marked in females; however, after 10 weeks the weight gain of female mice at 0.5% exceeds that of the controls. Generally, the dioxins cause a decrease in growth rate although dichlorodibenzodioxin, which has not been on test as long as the other compounds, does not appear to affect growth rate at the levels tested.

A summary of the results to date in the feeding studies is shown in Table 3. Despite the stimulation of growth in surviving animals, dioxane has caused an appreciable mortality in rats but has had little effect on mice. Chronic bronchopneumonia and chronic murine pneumonia above the background level were the major changes observ-

Table 2. Distribution of preneoplastic and neoplastic lesions in 15 mice treated with dioxane following DMBA initiation.

| Type of lesion | Number of mice with specific lesions | | | | | | |
|-----------------------------------|--------------------------------------|--------------|---------|------|--------|--------|-------------|
| | Skin | Nasal septum | Trachea | Lung | Spleen | Kidney | Pericardium |
| Hypertrophy and/or hyperplasia | 6 | | | 1 | | | |
| Papilloma | 2 | | | | | | |
| Carcinoma <i>in situ</i> | | | | 1 | | | |
| Squamous cell carcinoma | 2 | 1 | | 2 | | | |
| Bronchial adenomatoid lesion | | | | 3 | | | |
| Alveolar adenoma | | | | 4 | | | |
| Bronchiolar or alveolar carcinoma | | | | 3 | | | |
| Fibrosarcoma | 3 | | | 1 | 1 | | 1 |
| Undifferentiated sarcoma | 1 | | | 1 | | 1 | |
| Malignant lymphoma | | | | | | | |
| Lymphocyte type | | | 1 | 1 | 1 | 1 | |
| Reticulum cell sarcoma | | | | 1 | 1 | 1 | 1 |

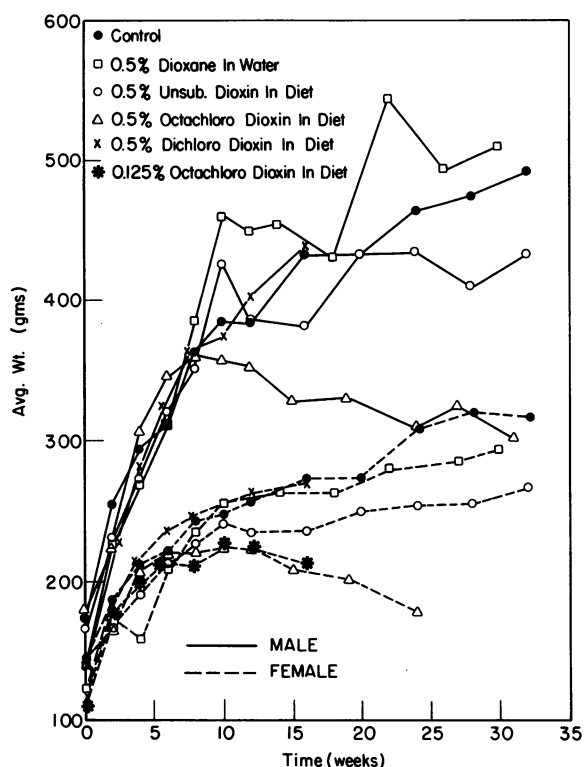


FIGURE 4. Average weight of treated female and male rats.

ed in the rats that have been examined. Appreciable mortality has also been observed in female mice that are receiving 1% unsubstituted dioxin. Hepatotoxicity was the major effect noted, but one mouse exhibited broncheolar mucosal hyperplasia. The rats that received this compound exhibited hepatotoxicity and chronic murine pneumonia.

Toxicity in animals fed octachlorodioxin was evidenced by both growth depression and mortality. A distinct difference in the susceptibility of the sexes to the compound was observed for mice and rats. Thus all male mice died by 10 and 8 weeks, respectively, in the 1% and 0.5% levels while, at 37 weeks, there were 5 female 1% survivors and 45 female survivors at 0.5%. On the other hand, all female rats died by 22 and 25 weeks, respectively, in the 1% and 0.5% groups while the last males died at 32 and 37 weeks. New test groups were set up at 0.25% for all animals and at 0.125% for female rats and male mice since these groups appeared to be more drug-sensitive. Even at these lower dose levels, all of the mice have died.

Rats and mice fed octachlorodibenzodioxin had changes ranging from early hepatotoxic

Table 3. Summary of dioxin oral administration data.

| Compound | Sex | Rats (35 per group) | | | | Mice (50 per group) | | | |
|----------------------------------|-----|---------------------|-----------|-----------------------|-------|---------------------|-----------|-----------------------|-------|
| | | Week of test | Survivors | Significant pathology | | Week of test | Survivors | Significant pathology | |
| | | | | Lung | Liver | | | Lung | Liver |
| Controls | M | 34 | 35 | | | 31 | 50 | | |
| | F | 34 | 35 | | | 31 | 50 | | |
| 1% Dioxane | M | 42 | 24 | 8/11 | 1/11 | 40 | 50 | | |
| | F | 42 | 20 | 3/5 | 2/5 | 43 | 49 | | |
| 0.5% Dioxane | M | 42 | 26 | 6/6 | 1/6 | 40 | 49 | | |
| | F | 42 | 32 | 3/3 | 1/3 | 43 | 49 | | |
| 1% Unsubstituted dibenzodioxin | M | 42 | 33 | 2/2 | 2/2 | 39 | 48 | | |
| | F | 42 | 32 | 1/2 | 2/2 | 39 | 29 | 3/14 | 7/14 |
| 0.5% Unsubstituted dibenzodioxin | M | 42 | 31 | 3/4 | 1/4 | 34 | 50 | | |
| | F | 42 | 35 | | | 39 | 49 | | |
| 1% Dichloro-dibenzodioxin | M | 17 | 35 | | | 17 | 49 | 0/1 | 0/1 |
| | F | 17 | 35 | | | 17 | 48 | 1/2 | 1/2 |
| 0.5% Dichloro-dibenzodioxin | M | 17 | 35 | | | 17 | 50 | | |
| | F | 17 | 35 | | | 17 | 49 | | |
| 1% Octachloro-dibenzodioxin | M | 32 | 0 | 1/5 | 5/5 | 10 | 0 | 0/5 | 5/5 |
| | F | 22 | 0 | 0/5 | 5/5 | 37 | 5 | 0/5 | 6/6 |
| 0.5% Octachloro-dibenzodioxin | M | 37 | 0 | 1/5 | 5/5 | 8 | 0 | 0/5 | 5/5 |
| | F | 25 | 0 | | | 37 | 45 | | |
| 0.25% Octachloro-dibenzodioxin | M | 17 | 28 | 1/2 | 2/2 | 17 | 1 | | |
| | F | 17 | 15 | 0/3 | 3/3 | 15 | 0 | 2/2 | 1/2 |
| 0.125% Octachloro-dibenzodioxin | M | — | — | — | — | 9 | 0 | | |
| | F | 17 | 30 | | | — | — | — | — |

lesions (diffuse vacuolar degeneration, fatty metamorphosis, megalocytosis, necrosis, and cholangiolar epithelial hyperplasia) to cirrhosis with disrupted lobule architecture, necrosis, fibrosis, parenchymal regeneration and cholangiolar proliferation. There were areas of bronchiolar mucosal hyperplasia in the lungs of two rats, and one of these rats also had bronchiolar adenomatoid lesions. In one mouse, there were bronchiolar mucosal hyperplasia and metaplasia and one bronchiolar carcinoma in which signs of early squamous change could be seen.

Discussion

The preliminary results reported here are by no means conclusive with regard to the carcinogenicity of the chlorinated dioxins. The oral studies indicate primarily hepatotoxicity, especially in the case of octachlorodibenzodioxin. A supplementary analysis report from Midwest Research Institute provides a possible explanation for some of

the toxicity observed. The batch of octachlorodibenzodioxin used in preparation of the diets was found to contain hexachlorodibenzodioxin at a level of less than 0.1%. A dietary level of 1% octachlorodibenzodioxin which contained this contaminant would provide a hexachlorodibenzodioxin intake of approximately 150 $\mu\text{g}/\text{day}$ for rats and 50 $\mu\text{g}/\text{day}$ for mice. Chronic toxicity values for this compound are not available. However, in preliminary experiments at Dow Chemical Co. (5), hexachlorodibenzodioxin at 100 $\mu\text{g}/\text{kg}/\text{day}$ for 10 days caused growth depression, liver pathology, and embryotoxicity in rats. Tests with animals receiving the first batch of octachlorodibenzodioxin will continue as long as there are survivors. Additional groups of animals at dietary levels of 0.5% and 0.25% octachlorodibenzodioxin shown on analysis not to contain the hexachlorodibenzodioxin contaminant have been started. After 6 weeks, many of the male mice at 0.5% have died, thereby indicating

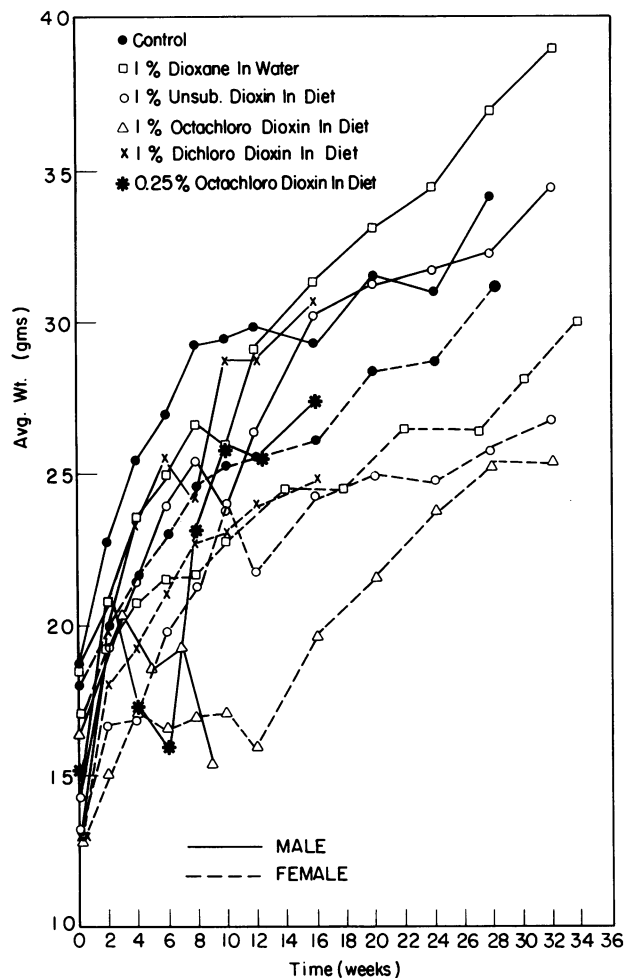


FIGURE 5. Average weight of treated female and male mice.

possible toxicity of the octachlorodibenzo-dioxin itself. A comparative feeding and intubation study is currently underway to determine if the octachloro causes a decrease in dietary intake.

The skin carcinogenesis assay for the nontoxic compounds is nearing completion, and no skin tumors have been observed in the complete carcinogenesis studies. The nature of the internal growths must be determined at necropsy. The fact that only male mice have exhibited skin tumors in the promotion studies can probably be attributed to the additional factor of wounding. As the

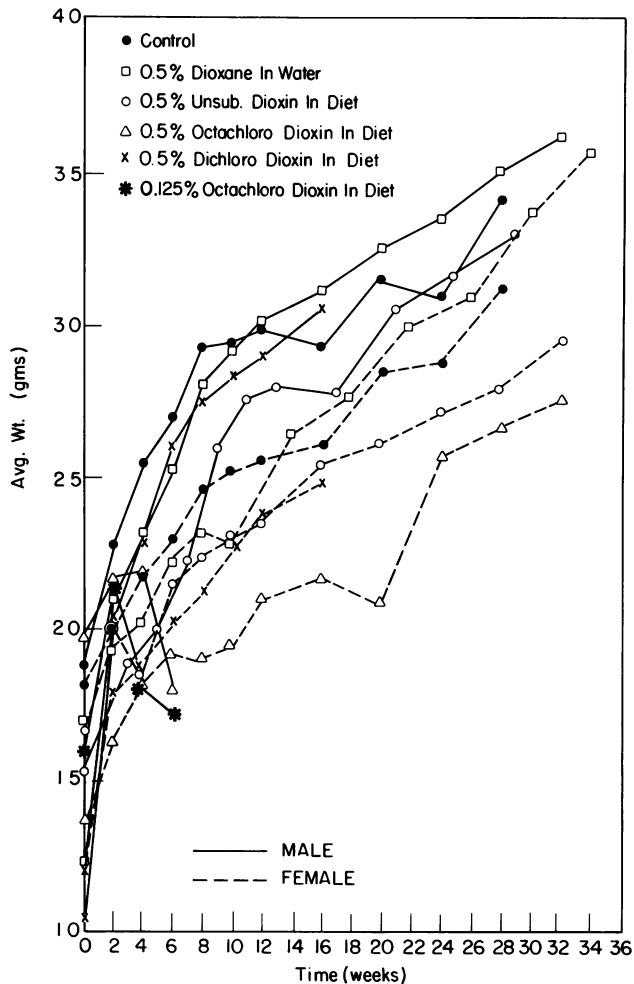


FIGURE 6. Average weight of treated female and male mice.

mice are housed in groups of 10, there is considerable fighting among the males, resulting in scars on the backs of the less aggressive animals. Boutwell (8) has shown that wound healing plays an integral role in the promotion of skin carcinogenesis, and fighting among males treated with unsubstituted and dichlorodibenzodioxin as well as acetone may have contributed to the promotion of skin tumors. No explanation can be given at this time for the lack of tumors in the octachlorodioxin group, since this compound was also dissolved in acetone.

The positive control group treated with

DMBA and croton oil responded as expected in terms of skin tumor promotion. The extent and variety of the lesions produced by DMBA and dioxane were unexpected, however, previous studies have shown dioxane to be a hepatocarcinogen (9) and to induce carcinomas in the nasal cavity of rats receiving dioxane in drinking water (10). No previous reports of dioxane as a promoting agent in skin carcinogenesis could be found. Because of the use of dioxane as a solvent in industry and in histology laboratories for tissue processing, the results obtained are of potential significance in relation to possible carcinogenic hazards to humans.

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